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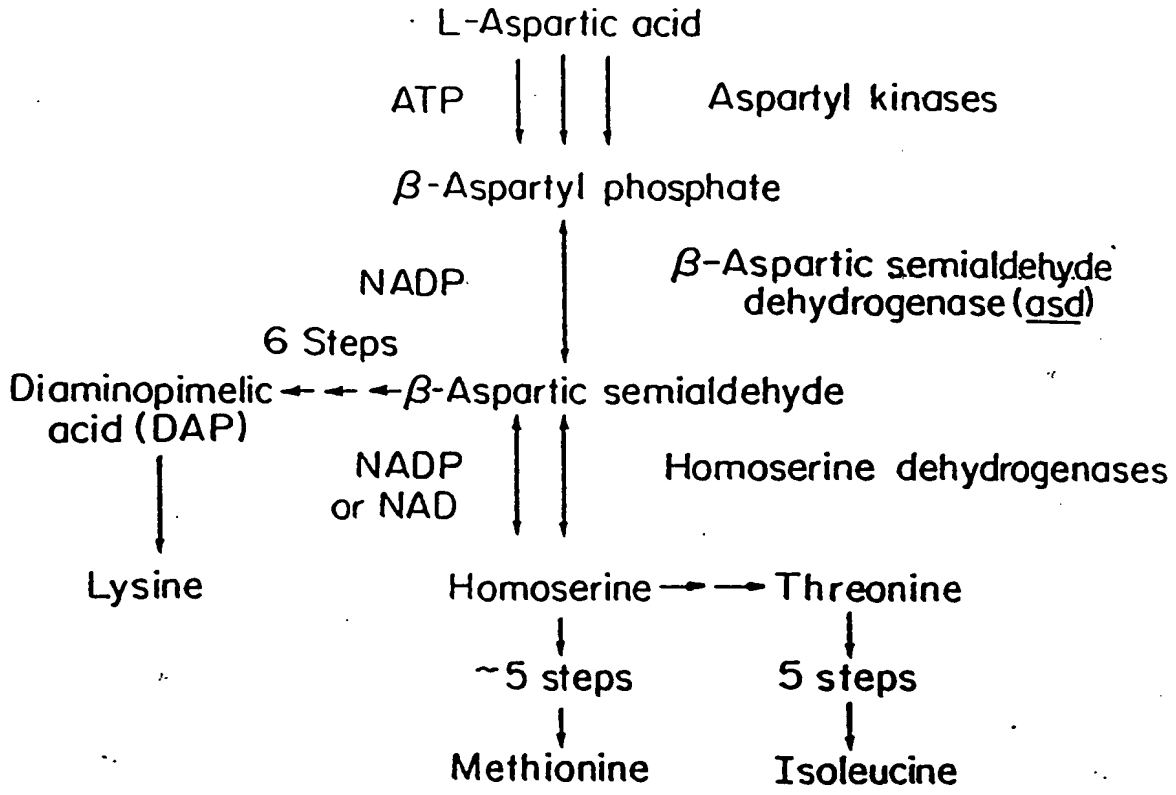

Figure 1

A

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  61 cattggccct caaacccctа attaggatca ataaaacagc gacggaaatg attcccttcc
 121 taacgcaaat tccctgataa tcgccactgg actttctgct tgcgcggtaa ggcaggataa
 181 gtcgcattac tgatggcttc gctatcattg attaatttca cttgcgactt tggctgcttt
 241 ttgtatggtg aaggatgcgc cacaggatac tggcgcgcat acacagcaca tctctttgca
 301 ggaaaaaaac gctatgaaaa atgttggttt tatcggctgg cgcggaatgg tcggctctgt
 361 tctcatgcaa cgcatggtag aggagcgcga tttcgacgct attcgccctg ttttcttttc
 421 tacctcccag tttggacagg cggcgcccac cttcggcgac acctccaccg gcacgctaca
 481 ggacgctttt gatctggatg cgctaaaagc gctcgatatc atcgtgacct gccagggcgg
 541 cgattatacc aacgaaattt atccaaagct gcgcgaaagc ggatggcagg gttactggat
 601 tgatgcggct tctacgctgc gcatgaaaga tgatgccatt attattctcg acccggtcaa
 661 ccaggacgtg attaccgacg gcctgaacaa tggcgtgaag acctttgtgg gcggtaactg
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 781 ggtatccgtc gcgacctatc aggccgcctc cggcggcggc gcgcgccata tgcgcgagct
 841 gttaacccag atgggtcagt tgtatggcca tgtcgccgat gaactggcga cgccgtcttc
 901 cgcaattctt gatattgaac gcaaagttac ggcattgacc cgcagcggcg agctgccggt
 961 tgataacttt ggcgtaccgc tggcgggaag cctgatcccc tggatcgaca aacagctcga
1021 taacggccag agccgcgaag agtggaaagg ccaggcggaa accaacaaga ttctcaatac
1081 tgcctctgtg attccggttg atggtttgtg tgtgcgcgtc ggcgcgctgc gctgtcacag
1141 ccaggcgttc accatcaagc tgaaaaaaga ggtatccatt ccgacggtgg aagaactgct
1201 ggcggcacat aatccgtggg cgaaagtggt gccgaacgat cgtgatatca ctatgcgcga
1261 attaaccccg gcggcggtga ccggcacgtt gactacgccg gttggtcgtc tgcgtaagct
1321 gaacatgggg ccagagttct tgtcggcgtt taccgtaggc gaccagttgt tatggggcgc
1381 cgccgagccg ctgcgtcgaa tgctgcgcca gttggcgtag tggctattgc agcgcttatc
1441 gggcctgcgt gtggttctgt aggccggata aggcgcgtca gcgccgccat ccggcgggga
1501 aatttgtgtt aaaccagggg tgcatcgtca ccctttttтt gcgtaataca ggagtaaacg
1561 cagatgtttc atttttatca ggagttaagc agagcattgg ctattcttta agggtagctt
1621 aatcccacgg gtattaagcc taacctgaag gtaggacgac gcagatagga tgcacagtgt
1681 gctgcgccgt tcaggtcaaa gaagtgtcac tacctgatgt tgaattggaa gatcc
```

B

```
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APTFGDTSTGTLQDAFDLDALKALDIIVTCQGGDYTNEIYPKLRESGWQGYWIDAASTLRMKDDAI
IILDPVNQDVITDGLNNGVKTFVGGNCTVSLMLMSLGGLFAHNLVDWVSVATYQAASGGGARHMRE
LLTQMGQLYGHVADELATPSSAILDIERKVTALTRSGELPVDNFGVPLAGSLIPWIDKQLDNGQSR
EEWKGQAETNKILNTASVIPVDGLCVRVGALRCHSQAFTIKLKKEVSIPTVEELLAAHNPWAKVVP
NDRDITMRELTPAAVTGTLTTPVGRLRKLNMGPEFLSAFTVGDQLLWGAAEPLRRMLRQLA
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Figure 2

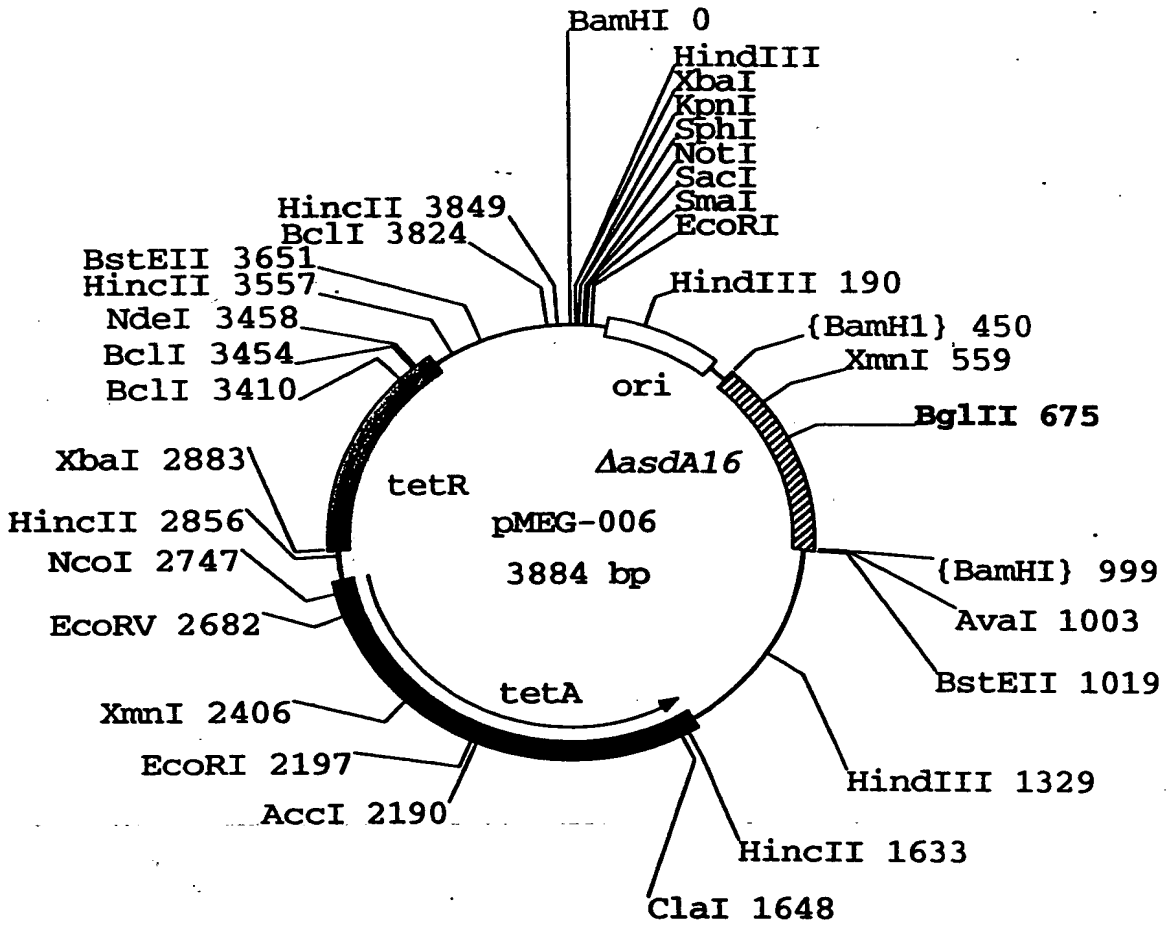
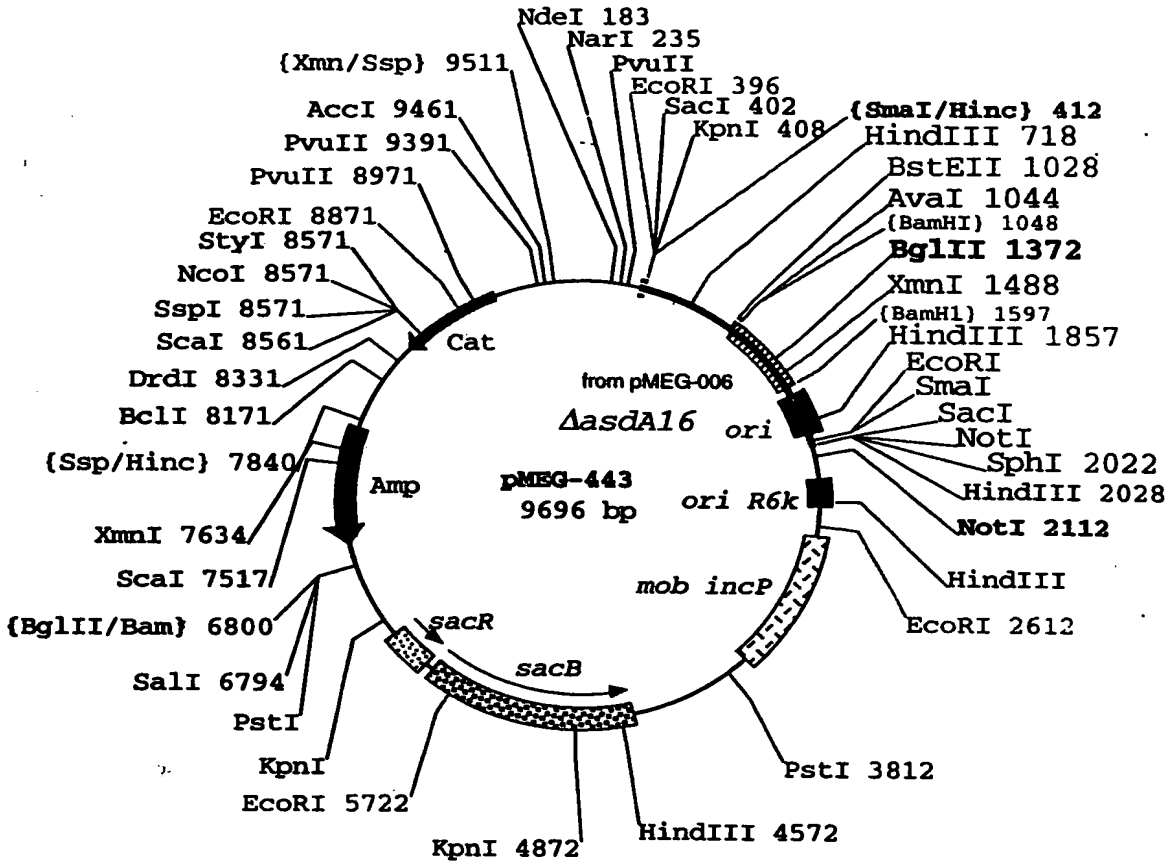


Figure 3



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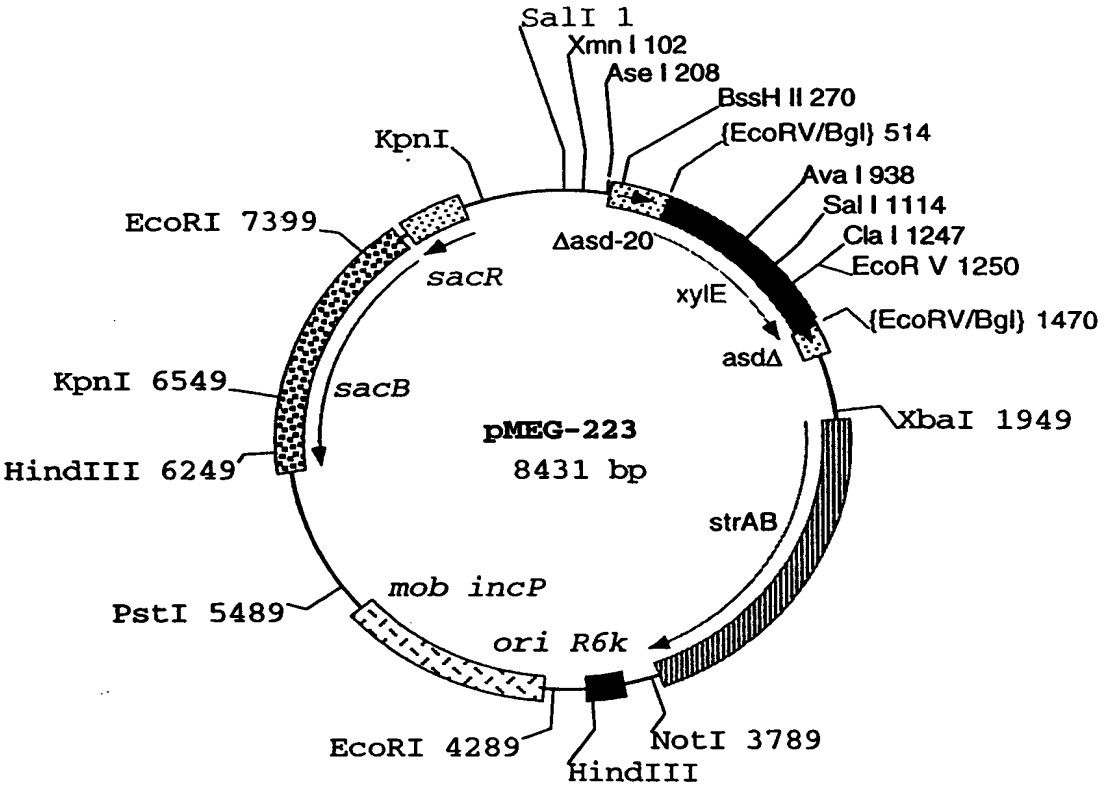

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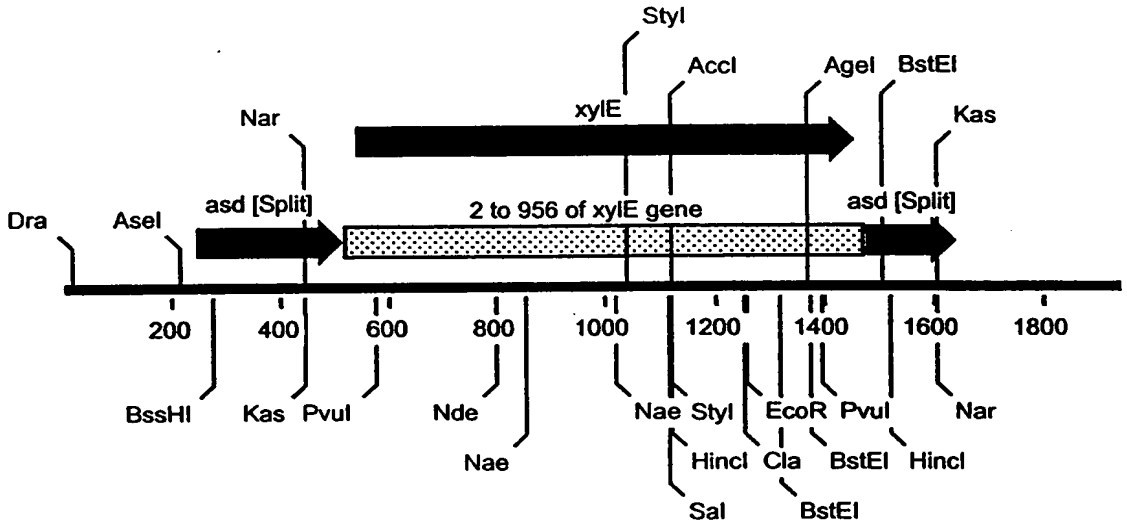
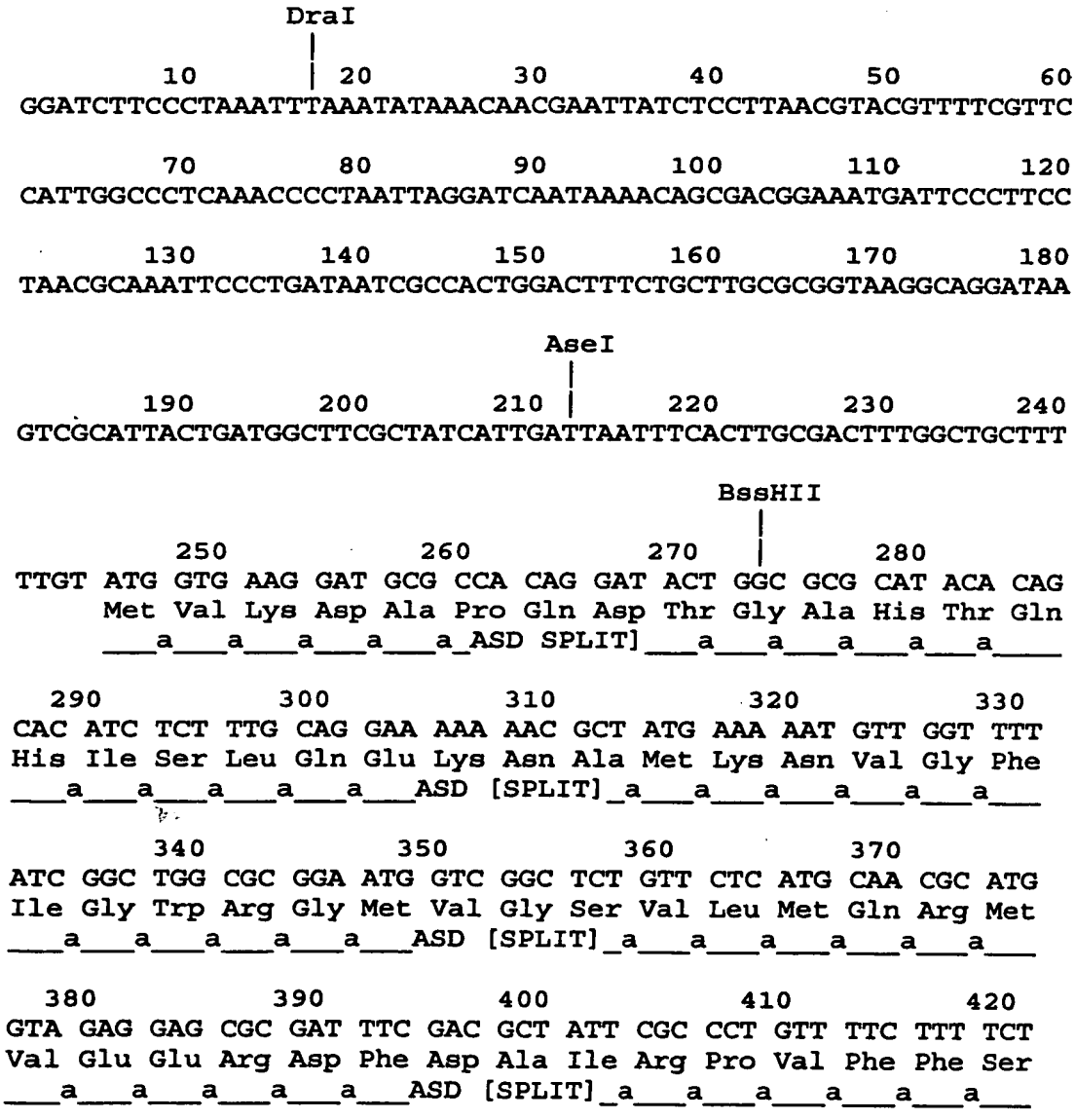


Figure 6A

Figure 6B



```
                                        NarI
                                         |
                                       KasI
                                        | |
         430                       440 | |      450                460
ACC TCC CAG TTT GGA CAG GCG GCG CCC ACC TTC GGC GAC ACC TCC
Thr Ser Gln Phe Gly Gln Ala Ala Pro Thr Phe Gly Asp Thr Ser
___a___a___a___a___a___ASD [SPLIT]_a___a___a___a___a___a___

  470           480           490           500           510
ACC GGC ACG CTA CAG GAC GCT TTT GAT CTG GAT GCG CTA AAA GCG
Thr Gly Thr Leu Gln Asp Ala Phe Asp Leu Asp Ala Leu Lys Ala
___a___a___a___a___a___ASD [SPLIT]_a___a___a___a___a___a___

        520       530         540           550           560
CTC GAT GATCTATGAAGAGGTGACGTC ATG AAC AAA GGT GTA ATG CGA CCG
Leu Asp                        Met Asn Lys Gly Val Met Arg Pro
__a___                         ___c___c___c_XYLE__c___c___c___

              PvuII
                |
       570      |      580           590           600
GGC CAT GTG CAG CTG CGT GTA CTG GAC ATG AGC AAG GCC CTG GAA
Gly His Val Gln Leu Arg Val Leu Asp Met Ser Lys Ala Leu Glu
___c___c___c___c___c___c___XYLE____c___c___c___c___c___c___

610           620           630           640           650
CAC TAC GTC GAG TTG CTG GGC CTG ATC GAG ATG GAC CGT GAC GAC
His Tyr Val Glu Leu Leu Gly Leu Ile Glu Met Asp Arg Asp Asp
___c___c___c___c___c___c___XYLE____c___c___c___c___c___c___

       660           670           680           690
CAG GGC CGT GTC TAT CTG AAG GCT TGG ACC GAA GTG GAT AAG TTT
Gln Gly Arg Val Tyr Leu Lys Ala Trp Thr Glu Val Asp Lys Phe
___c___c___c___c___c___c___XYLE____c___c___c___c___c___c___

700           710           720           730           740
TCC CTG GTG CTA CGC GAG GCT GAC GAG CCG GGC ATG GAT TTT ATG
Ser Leu Val Leu Arg Glu Ala Asp Glu Pro Gly Met Asp Phe Met
___c___c___c___c___c___c___XYLE____c___c___c___c___c___c___

       750           760           770           780
GGT TTC AAG GTT GTG GAT GAG GAT GCT CTC CGG CAA CTG GAG CGG
Gly Phe Lys Val Val Asp Glu Asp Ala Leu Arg Gln Leu Glu Arg
___c___c___c___c___c___c___XYLE____c___c___c___c___c___c___
```

Figure 6B
(Con't.)

```
                NdeI
                 |
790              800             810             820             830
GAT CTG ATG GCA TAT GGC TGT GCC GTT GAG CAG CTA CCC GCA GGT
Asp Leu Met Ala Tyr Gly Cys Ala Val Glu Gln Leu Pro Ala Gly
___c___c___c___c___c___c___XYLE____c___c___c___c___c___c___

                          NaeI
                           |
       840             850 |           860             870
GAA CTG AAC AGT TGT GGC CGG CGC GTG CGT TCC AGG CCC TCC GGG
Glu Leu Asn Ser Cys Gly Arg Arg Val Arg Ser Arg Pro Ser Gly
___c___c___c___c___c___c___XYLE____c___c___c___c___c___c___

880             890             900             910             920
CAT CAC TTC GAG TTG TAT GCA GAC AAG GAA TAT ACT GGA AAG TGG
His His Phe Glu Leu Tyr Ala Asp Lys Glu Tyr Thr Gly Lys Trp
___c___c___c___c___c___c___XYLE____c___c___c___c___c___c___

       930             940             950             960
GGT TTG AAT GAC GTC AAT CCC GAG GCA TGG CCG CGC GAT CTG AAA
Gly Leu Asn Asp Val Asn Pro Glu Ala Trp Pro Arg Asp Leu Lys
___c___c___c___c___c___c___XYLE____c___c___c___c___c___c___

970             980             990             1000            1010
GGT ATG GCG GCT GTG CGT TTC GAC CAC GCC CTC ATG TAT GGC GAC
Gly Met Ala Ala Val Arg Phe Asp His Ala Leu Met Tyr Gly Asp
___c___c___c___c___c___c___XYLE____c___c___c___c___c___c___

        NaeI                            StyI
         |                               |
      1020            1030            1040|            1050
GAA TTG CCG GCG ACC TAT GAC CTG TTC ACC AAG GTG CTC GGT TTC
Glu Leu Pro Ala Thr Tyr Asp Leu Phe Thr Lys Val Leu Gly Phe
___c___c___c___c___c___c___XYLE____c___c___c___c___c___c___

1060            1070            1080            1090            1100
TAT CTG GCC GAA CAG GTG CTG GAC GAA AAT GGC ACG CGC GTC GCC
Tyr Leu Ala Glu Gln Val Leu Asp Glu Asn Gly Thr Arg Val Ala
___c___c___c___c___c___c___XYLE____c___c___c___c___c___c___
```

Figure 6B
(Con't.)

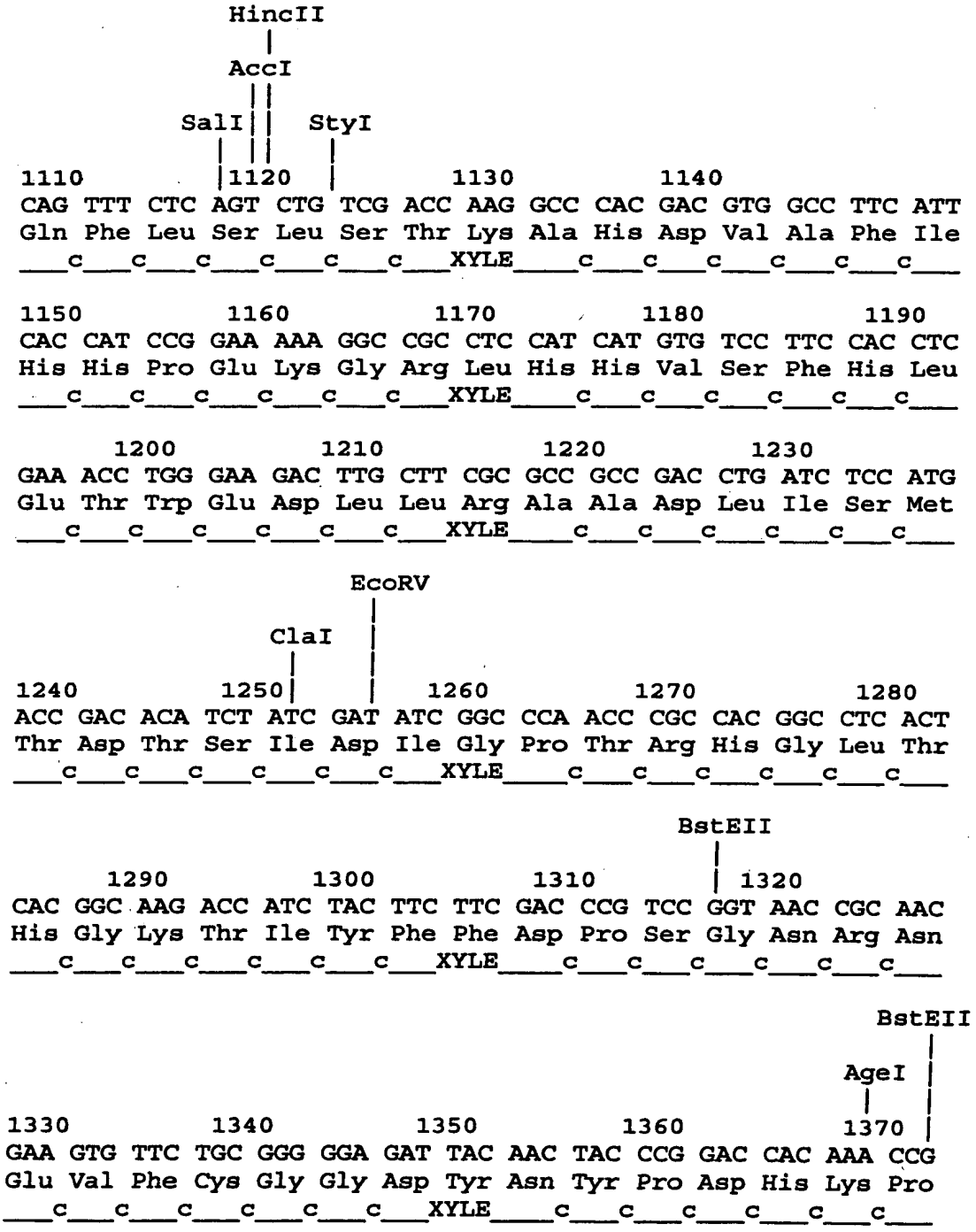


Figure 6B
(Con't.)

```
                                    PvuII
                                      |
      1380          1390            |      1400          1410
GTG ACC TGG ACC ACC GAC CAG CTG GGC AAA GCC TTC TTT TAC CAC
Val Thr Trp Thr Thr Asp Gln Leu Gly Lys Ala Phe Phe Tyr His
___c___c___c___c___c___c___XYLE____c___c___c___c___c___c___

1420          1430          1440          1450
GAC CGC ATT CTC AAC GAA CGA TTC ATG ACC GTG CTG ACC
Asp Arg Ile Leu Asn Glu Arg Phe Met Thr Val Leu Thr
___c___c___c___c___c___XYLE____c___c___c___c___c___

                                                          BstEII
                                                            |
1460      1470          1480          1490          1500  |
TGATGGTCCGGAGATC ATC ACT ATG CGC GAA TTA ACC CCG GCG GCG GTG
                 Ile Thr Met Arg Glu Leu Thr Pro Ala Ala Val
                 ___b___b___b___ASD [SPLIT]_b___b___b___b___

            HincII
              |
  1510        | 1520          1530          1540          1550
ACC GGC ACG TTG ACT ACG CCG GTT GGT CGT CTG CGT AAG CTG AAC
Thr Gly Thr Leu Thr Thr Pro Val Gly Arg Leu Arg Lys Leu Asn
___b___b___b___b___b___ASD [SPLIT]_b___b___b___b___b___b___

          1560          1570          1580          1590
ATG GGG CCA GAG TTC TTG TCG GCG TTT ACC GTA GGC GAC CAG TTG
Met Gly Pro Glu Phe Leu Ser Ala Phe Thr Val Gly Asp Gln Leu
___b___b___b___b___b___ASD [SPLIT]_b___b___b___b___b___b___

        NarI
          |
        KasI
          ||
  1600    ||    1610          1620          1630          1640
TTA TGG GGC GCC GCC GAG CCG CTG CGT CGA ATG CTG CGC CAG TTG
Leu Trp Gly Ala Ala Glu Pro Leu Arg Arg Met Leu Arg Gln Leu
___b___b___b___b___b___ASD [SPLIT]_b___b___b___b___b___b___

       1650      1660      1670      1680
GCG TAGTGGCTATTGCAGCGCTTATCGGGCCTGCGTGTGG
Ala
___
```

Figure 6B
(Con't.)

```
      1690        1700        1710        1720        1730        1740
TTCTGTAGGCCGGATAAGGCGCGTCAGCGCCGCCATCCGGCGGGGAAATTTGTGTTAAAC

      1750        1760        1770        1780        1790        1800
CAGGGGTGCATCGTCACCCTTTTTTTGCGTAATACAGGAGTAAACGCAGATGTTTCATTT

      1810        1820        1830        1840        1850        1860
TTATCAGGAGTTAAGCAGAGCATTGGCTATTCTTTAAGGGTAGCTTAATCCCACGGGTAT

      1870        1880        1890        1900        1910        1920
TAAGCCTAACCTGAAGGTAGGACGACGCAGATAGGATGCACAGTGTGCTGCGCCGTTCAG

      1930        1940        1950        1960
GTCAAAGAAGTGTCACTACCTGATGTTGAATTGGAAGATCC
```

Figure 6B
(Con't.)

Nucleotide sequences of trc promoter/operator and MCS

MCS: *Nco*I *Eco*RI --------*Hin*dIII

pYA3098, pYA3148, pYA3332, pYA3333, pYA3334,

pYA3336, pYA3339, pYA3340, pYA3341, pYA3342

-35
5′ ATTCTGAAATGAGCTGTTGACAATTAATCATCCGGCTC

-10
GTATAATGTGTGGAATTGTGAGCGGATAACAATTTCACAC

SD Ncol EcoRI SmaI
AGGAAACAGACC ATG G[illegible]GC AAT TCC CGG GGA
Met Gly Ile Arg Asn Ser Arg Gly

BamHI SalI PstI HindIII
TCC GTC GAC CTG CAG CCA AGC TCC CAA GCT T 3′
Ser Val Asp Leu Gln Pro Ser Ser Gln Ala

Figure 7

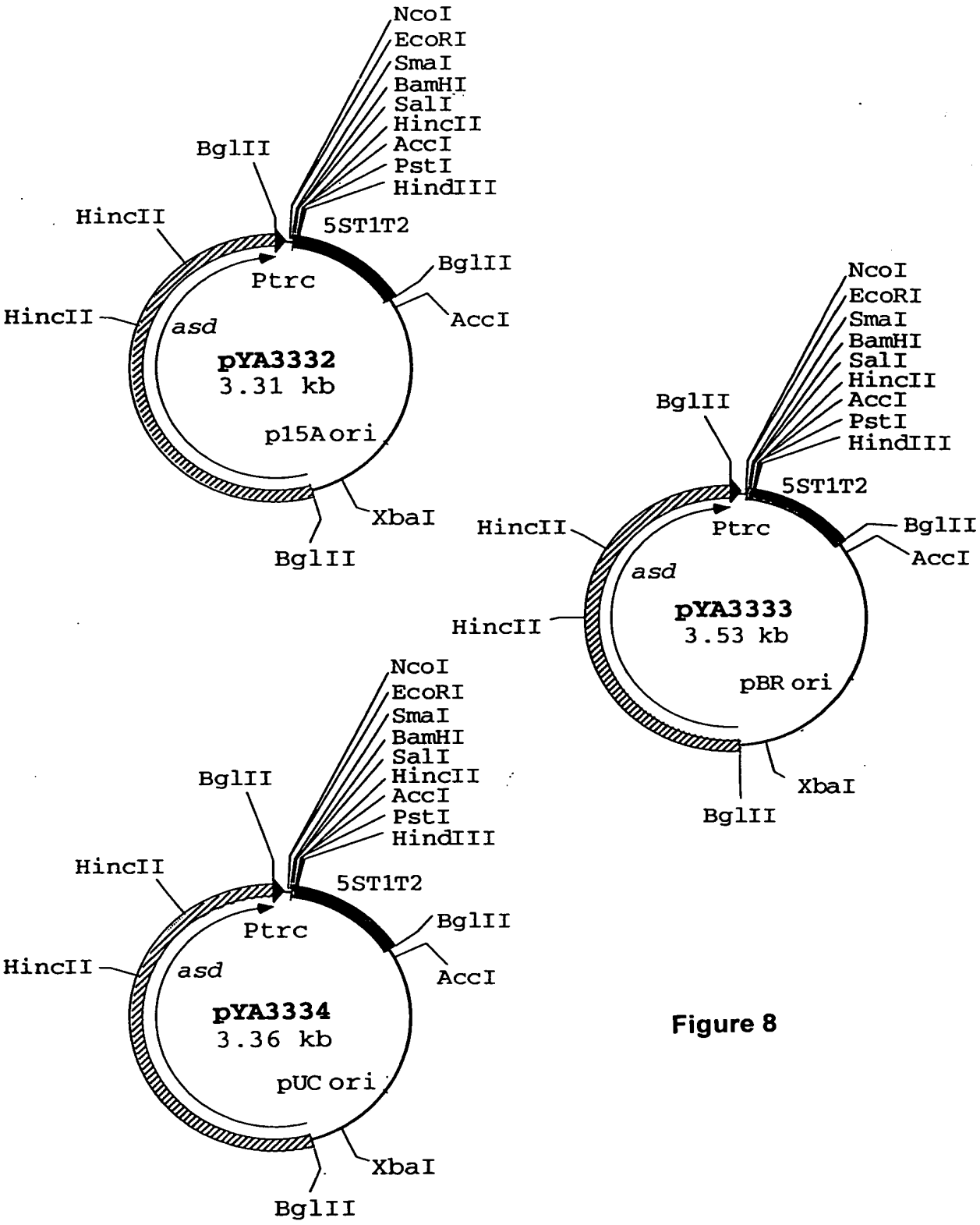


Figure 8

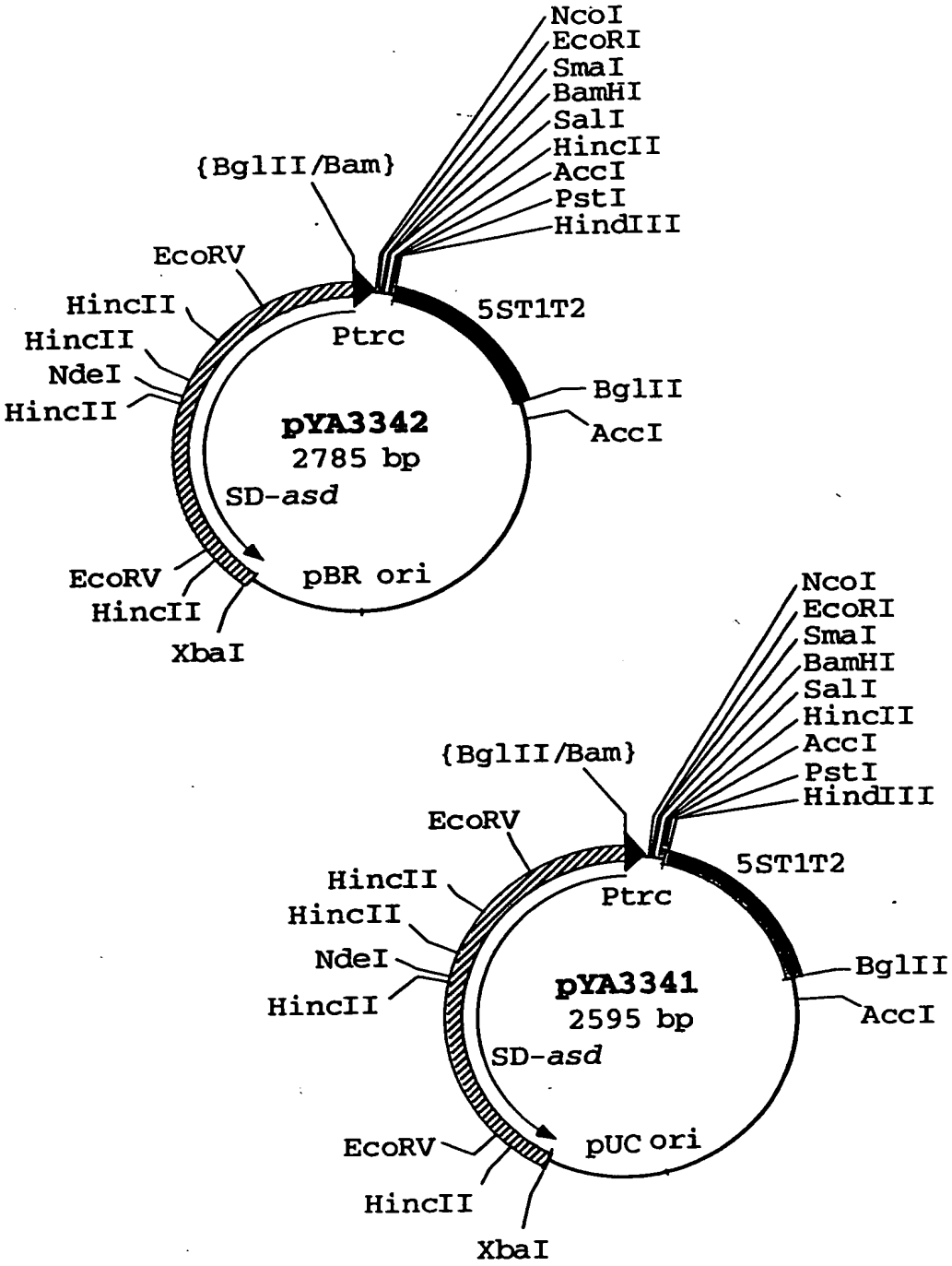
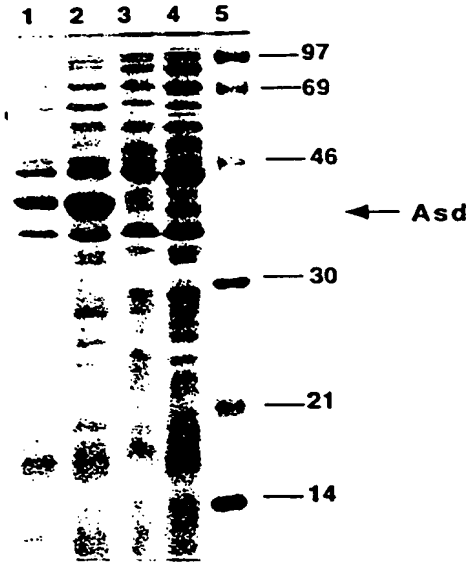


Figure 9

Level of Asd sythesized in recombinant *S. typhimurium* strains with different Asd+ plasmids



Cell lysates of *S. typhimurium* χ4550 with pYA3333 (lane 1), pYA3334 (lane 2), pYA3342 (lane 3) and pYA3341 (lane 4). Lane 5 contains molecular weight markers. The arrow indicates Asd protein band.

Figure 10

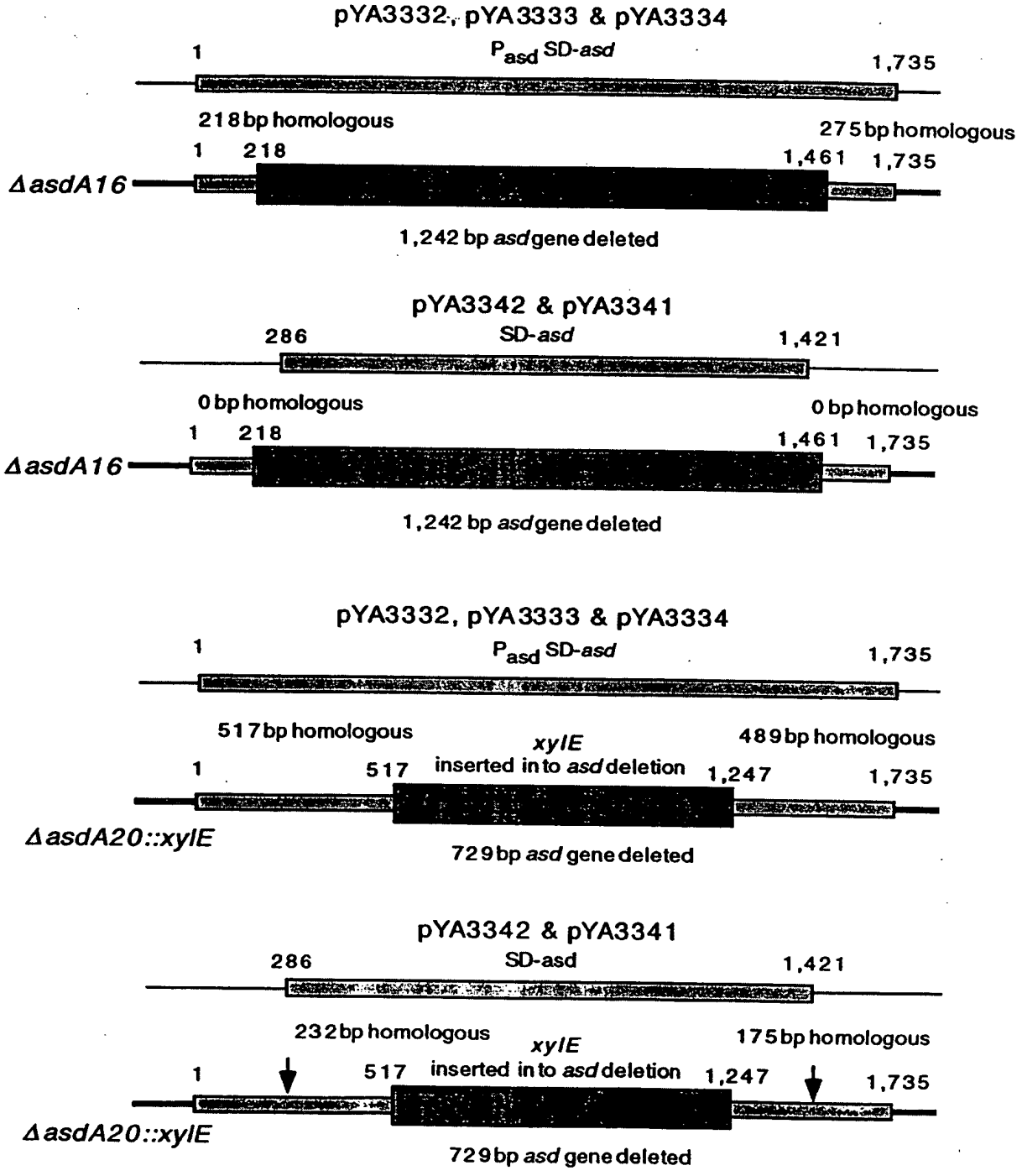


Figure 11

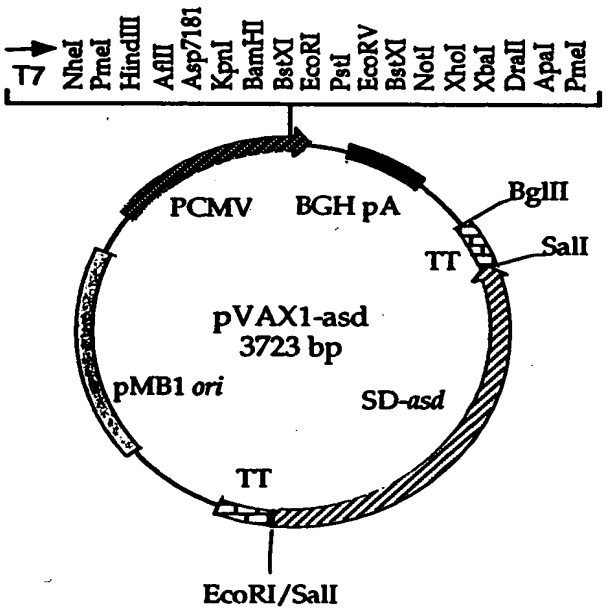


Figure 12